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Methods of Evaluating Oocytes for Storage or Reproductive Treatments

Background of the Invention

A woman's fertility begins to decline around age 28, and declines more rapidly after age 35. Declining egg viability is one of the major factors contributing to compromised fertility in women. In addition to the natural aging process, chemotherapy and radiation treatment for cancer are also known to affect a woman's ability to produce healthy eggs. By freezing eggs before cancer treatment or earlier in life when egg quality is higher, a woman may be able to retain her ability to become pregnant and have a child many months or even years later.

Summary of the Invention

The present invention is based, in part, on the discovery that by obtaining the first polar body of an unfertilized oocyte, the quality of the oocyte can be evaluated prior to storage. Such analysis allows oocytes that are more likely to give rise to a healthy embryo to be stored while those oocytes less likely to give rise to a healthy embryo can be discarded. In addition, the methods provide an opportunity for subjects looking to freeze their eggs to evaluate the quality of those eggs prior to storage and make a decision about whether more eggs should be harvested.

Accordingly, in one aspect, the invention features a method of determining the suitability of at least one unfertilized oocyte for storage. The method includes the steps of: (a) providing an analysis of the first polar body from an unfertilized oocyte that indicates the desirability of storing that unfertilized oocyte; and (b) storing or discarding the unfertilized oocyte based upon the results obtained from the analysis of the polar body. In one embodiment, the method further includes providing or retrieving one or more oocytes from the oocyte donor based upon the analysis. The party that provides or retrieves the one or more oocytes can be ,e.g., the same party that performs steps (a) and/or (b) or can be a different party.

In one embodiment, the unfertilized oocyte is a human oocyte. In another embodiment, the unfertilized oocyte is a non-human oocyte, e.g., the unfertilized oocyte is taken from a prize animal.

In one embodiment, the analysis includes a determination of whether the polar body has one or more chromosomes above or below the normal chromosome number. In another embodiment, based upon the analysis that the unfertilized oocyte associated with a polar body has one or more chromosomes above or below the normal number and/or is lacking or has more than one copy of a particular chromosome or chromosomes, the unfertilized oocyte is discarded. In another embodiment, based upon the analysis that the unfertilized oocyte has one or more chromosomes aboveor below the normal number and/or is lacking or has more than one copy of a particular chromsome or chromosomes, the method further includes retrieving one or more oocytes from the donor. In another embodiment, based upon the analysis that the polar body that the unfertilized oocyte associated with a polar body has a normal number of chromosomes and/or has one copy of a particular chromosome or chromosomes, the unfertilized oocyte is stored. In another embodiment, the method can further include using the stored oocyte in a fertility or reproductive treatment, e.g. *in vitro* fertilization.

In one embodiment, the analysis indicates the absence or presence of one or more chromosomes, e.g. one or more of chromosomes 1-22, above or below the normal number of chromosomes in the polar body, thereby indicating that the oocyte is unlikely to give rise to a healthy embryo. In one embodiment, the analysis indicates one or more of: the presence of more than one chromosome 21 in the oocyte, and is indicative of a risk for Down's Syndrome; the presence of more than one chromosome 13 in the oocyte, and is indicative of a risk for Patau syndrome; the presence of more than one chromosome 18 in the oocyte, and is indicative of a risk for Edwards syndrome; the presence of more than one chromosome 16 in the oocyte, and is indicative of a risk for abnormal fetal development and/or miscarriage; the presence of more than one chromosome 22 in the oocyte, and is indicative of a risk for abnormal fetal development and/or miscarriage; the absence of chromosome 5 in the oocyte, and is indicative of a risk for Cri du Chat; the presence of more than one X chromosome in the oocyte, and is indicative of a risk for Klinefelter's syndrome; the presence of more than one X chromosome in the oocyte, and is indicative of a risk for Triple X syndrome; and the absence of chromosome X in the oocyte, and is indicative of a risk for Turner's syndrome. In one embodiment, the analysis indicates that the polar body has one or more chromosomes above or below the normal number of

chromosomes, e.g. one or more of the chromosomes described herein, and the oocyte is discarded.

In another embodiment, the analysis of a polar body includes a determination of whether the polar body has one or more structural chromosomal abnormalities. In one embodiment, when the analysis indicates the presence of one or more structural chromosomal abnormalities, the oocyte is discarded. In another embodiment, when the analysis indicates that the unfertilized oocyte has one or more structural chromsomal abnormalities, the method further includes retrieving one or more oocytes from the donor. In other embodiments, when the analysis indicates that one or more structural abnormalities are absent, the oocyte is stored.

In some embodiments, the analysis indicates whether a translocation is present or absent from the chromosomal material of the polar body. In some embodiments, the analysis can indicate the absence or presence of a translocation at one or more of chromosomes: 13, 14, 15, 21 or 22 of the polar body, the presence of which is indicative, e.g., of a risk for Robertsonian translocations. In some embodiments, when the analysis indicates the presence of one or more translocations, e.g. a translocation at one or more of chromosomes 13, 14, 15, 21 or 22, the associated oocyte is discarded. In another embodiment, when the analysis indicates the presence of one or more translocations, e.g. a translocation at one or more of chromosomes 13, 14, 15, 21 or 22, the method further includes retrieving one or more oocytes from the donor. In yet another embodiment, the analysis indicates that the polar body lacks one or more translocations, e.g., lacks a translocation at one or more of chromosomes 13, 14, 15, 21 or 22, and the associated oocyte is stored. In another embodiment, the analysis indicates whether a reciprocal translocation is present or absent from the chromosomal material of the polar body. In another embodiment, when the analysis indicates the presence of one or more reciprocal translocations in the chromosomal material of the polar body, the associated oocyte is discarded. In another embodiment, when the analysis indicates the presence of one or more reciprocal translocations, the method further includes retrieving one or more oocytes from the donor. In another embodiment, when the analysis indicates that the polar body lacks one or more reciprocal translocations, the associated oocyte is stored.

In one embodiment, the analysis of the first polar body includes a determination of whether there is a risk that the oocyte may carry one or more genetic disorders, e.g. one or more single-gene disorders. In one embodiment, the analysis of the first polar body includes a determination of whether one or more mutations (e.g., insertions, deletions and/or substitutions) in a gene or genes, e.g., a gene or genes associated with a genetic disorder, are present or absent. In some embodiments, the analysis indicates the absence or presence of a mutation or mutations in one or more genes associated with a disorder, e.g. in one or more of: a gene encoding CFTR, a gene encoding dystrophin, a Beta Thalassemia gene, a gene encoding Factor VIII, a gene encoding Factor IX, Tay-Sachs gene, a survival motor neuron (SMN) gene, and a HD gene.

In another embodiment, the analysis of the first polar body indicates the absence or presence of a genotype associated with one or more of the following genetic disorders: Adenoleukodystrophy, Amyotrophic Lateral Sclereosis (ALS), Becker Muscular Dystrophy, Beta Thalassemia, Cerebellar Ataxia, Charcot-Marie-Tooth Disease, Chondrodysplasia Aganglionic Megacolon, Conradi-Hunnerman Syndrome, Cystic Fibrosis, Duchenne Muscular Dystrophy, Hemophilia A or B, Huntington's Disease, Fragile X Syndrome, Glycogen Storage Disease, Hirschsprung Disease, Icthyosis, Lesch Nyhan, Myopathies, Polycystic Ovary Syndrome, Restenosis Pigmentosa, Sickle ell Anemia, Tay-Sachs Disease, and Von Willebran Disease. In another embodiment, the analysis of the first polar body indicates the presence or absence of one or more mutations in a gene or genes for which the oocyte donor is a heterozygous carrier, e.g. a heterozygous carrier of a gene or genes associated with a disorder.

In another embodiment, the analysis of the first polar body indicates the absence or presence of one or more insertion, deletion or substitution of a gene or genes of interest, and the decision to store or discard the associated oocyte is based upon the analysis. In one embodiment, where the analysis indicates that the oocyte donor is a heterozygous carrier of a genetic disorder, and the first polar body is found to lack one or more insertions, deletions, or substitutions associated with the genetic disorder, then the associated oocyte is discarded. In another embodiment, where the analysis indicates that the oocyte donor is a heterozygous carrier of a genetic disorder, and the first polar body is found to lack one or more insertions, deletions, or substitutions associated with the genetic disorder, the method further includes retrieving one or more oocytes from the donor. In another embodiment, where the analysis indicates that the oocyte donor is heterozygous

carrier of a genetic disorder, and the first polar body is found to have one or more insertions, deletions, or substitutions associated with the genetic disorder, the associated oocyte is stored.

In another embodiment, the analysis of the suitability of the oocyte for storage includes a determination regarding the morphology of the first polar body, e.g. according to one or more of the following morphological criteria: shape, surface smoothness, fragmentation, and size of the perivitalline space. In one embodiment, the analysis indicates that the first polar body has one or more morphological features associated with poor embryo quality and/or poor embryo viability, and the associated oocyte is discarded. In another embodiment, the analysis indicates that the first polar body has one or more morphological features associated with poor embyo quality and/or poor embryo viability, and the method further includes retrieving one or more oocytes from the donor. In another embodiment, the analysis indicates that the first polar body has one or more morphological features associated with good embryo quality and/or viability, and the associated oocyte is stored, e.g. for later use in a fertility or reproductive treatment.

In one embodiment, the analysis indicates that at least one unfertilized oocyte is determined suitable for storage, and that unfertilized oocyte is stored, e.g., by freezing or drying the unfertilized oocyte. In another embodiment, the method further includes treating the unfertilized oocyte to cause it to enter a dormant state prior to storing the unfertilized oocyte. In one embodiment, the unfertilized oocyte that is to be stored is treated by freezing, drying or both, e.g. by plunge freezing, vacuum drying, air drying, freezer drying, or a combination thereof. In one embodiment, the unfertilized oocyte is stored by freezing and the oocyte is frozen to a cryogenic temperature. In another embodiment, the oocyte is stored by freezing and the oocyte is frozen by cooling the oocyte at a rate between 0.3° C and 6° C per minute to a final temperature that is at least -50° C. In another embodiment, the oocyte is stored by freezing and the oocyte is frozen by cooling the oocyte at a rate between 0.3° C and 3° C per minute to a final temperature that is between –50° C and -10° C.

In another embodiment, the oocyte is stored by drying and the oocyte is dried to a level sufficient to permit dry storage. In another embodiment, the oocyte is stored by freeze drying. In another embodiment the oocyte is stored by vacuum and/or convective drying.

In one embodiment, the oocyte is stored and the method further includes, prior to storage, introducing into the unfertilized oocyte, e.g. by microinjection into the cytoplasm, a protective agent. The protective agent can have one or more of the following properties: (i) the agent includes a sugar or combination of sugars; (ii) the agent is substantially non-permeating; and (iii) it maintains the viability of the oocyte such that it can be stored in a temporarily dormant state and restored to an active state. In one embodiment, the oocyte is stored and the method includes contacting the oocyte to be stored with an extracellular protective agent, e.g. a protective agent that is substantially non-permeating with respect to mammalian cell membranes and that stabilizes the cell membrane of the oocyte. In another embodiment, when the oocyte is stored with a protective agent, the protective agent includes one or more sugars selected from the group consisting of: sucrose, trehalose, fructose, dextran, and raffinose. In another embodiment, when the oocyte is stored with a protective agent, the protective agent includes at least one sugar selected from the group consisting of: glucose, sorbitol, mannitol, lactose, maltose, and stachyose. In other embodiments, when the oocyte is stored with a protective agent, the protective agent includes at least one sugar with a glass transition temperature greater than -50° C or at least one sugar with a glass transition temperature greater than -30° C. In another embodiment, when the oocyte is stored with a protective agent, the protective agent includes at least one sugar with a molecular weight greater than 120 daltons. In another embodiment, when the oocyte is stored with a protective agent, the protective agent comprises at least one sugar with a glass transition temperature greater than -30° C and a molecular weight greater than 120 daltons. In another embodiment, when the oocyte is stored with a protective agent, the protective agent comprises a glycolipid and/or a glycoprotein that includes at least one sugar moiety derived from a sugar with a glass transition temperature greater than -50° C.

In another embodiment, when the oocyte is stored with a protective agent that includes a sugar, the cytoplasmic concentration of sugar is less than or equal to about 1.0 M after the introduction of the sugar into the oocyte cytoplasm, but prior to causing the oocyte to enter a dormant state. In an alternate embodiment, when the oocyte is stored with a protective agent that includes sugar, the cytoplasmic concentration of sugar is less than or equal to about 0.2 M following introduction of the sugar into the oocyte cytoplasm, but prior to causing the oocyte to enter a dormant state. In another embodiment, when the oocyte is stored with an extracellular

protective agent, the extracellular protective agent includes a sugar. In another embodiment, when the oocyte is stored with an extracellular protective agent, the oocyte is maintained in a liquid medium with an extracellular concentration of sugar that is less than or equal to either about 1.0 M, or alternatively about 0.2 M, following dilution into the liquid medium. In another embodiment, when the oocyte is stored with an extracellular protective agent, the oocyte is maintained on a solid medium with an extracellular concentration of sugar that is less than or equal to either about 1.0 M, or alternatively to about 0.2 M, following administration to the cell.

In other embodiments, when the oocyte is stored with a protective agent and/or an extracellular protective agent, a penetrating cryoprotectant mixture is added to the protective agent, e.g. any of the protective agents described above.

In another embodiment, the oocyte is stored and the method includes introducing into the unfertilized oocyte, e.g. by microinjection into the cytoplasm, a protective agent and treating the unfertilized oocyte to cause it to enter a dormant state prior to storing the unfertilized oocyte.

In other embodiments, the oocyte is stored and the method further includes treating the stored oocyte to restore the unfertilized oocyte to an active state. In one embodiment, the treatment to restore the unfertilized oocyte to an active state includes thawing the oocyte, rehydrating the oocyte or both. For example, the oocyte can be cultured in a hypertonic medium having an osmolarity greater than 300 mosm to restore the oocyte to an active state.

In another embodiment, the method includes a step of entering into a database the results of the analysis of the suitability of the unfertilized oocyte. In another embodiment, the step further includes correlating the results with the location of the associated unfertilized oocyte, e.g. the location of the associated oocyte in storage. In another embodiment, the method further includes entering into a database a code or value that indicates the suitability of the unfertilized oocyte for use in a fertility or reproductive treatment and entering a code, value, or location that correlates the suitability code or the suitability value with the unfertilized oocyte, or the location of the unfertilized oocyte. In another embodiment, the method further includes (a) accessing the database containing the analysis of the first polar body that indicates the suitability of the associated unfertilized oocyte for use in fertilization or reproductive treatment, or (a) accessing the database containing a value or code (generated by analyzing a first polar body) indicating the

suitability of a stored oocyte for use in a fertilization or reproductive treatment; and (b) retrieving the oocyte from storage if the analysis of the polar body in the database, or alternatively, if the code or value assigned to the oocyte in the database, indicates that the oocyte is suitable for use in the fertility or reproductive treatment. In a related embodiment, step (a), accessing the database, is performed by one party, and step (b), retrieving the oocyte from storage, is performed by second party. In another embodiment, both steps (a) and (b) are performed by the same party.

In one embodiment, the method includes providing an analysis of the first polar body from a second unfertilized oocyte, e.g., a second unfertilized oocyte from the same donor, that indicates the desirability of storing the second unfertilized oocyte; and storing or discarding the second oocyte based upon the results provided in the analysis of the polar body. The method can further include repeating the method on a third, fourth, fifth, etc. oocyte, e.g., from the same or a different donor.

In another aspect, the invention features a method of determining the suitability of at least one unfertilized oocyte for storage. The method includes the steps of: (a) providing the first polar body associated with an unfertilized oocyte; (b) evaluating the first polar body to determine the desirability of storing the unfertilized oocyte; and (c) storing or discarding the unfertilized oocyte based upon the evaluation of the polar body.

In one embodiment, all steps of the method are performed by one party. In another embodiment, steps (a) and (c) are performed by a first party and step (b) is performed by a second party. In another embodiment, steps (a) and (b) are performed by a first party and step (c) is performed by a second party. In yet another embodiment, step (a) is performed by a first party and steps (b) and (c) are performed by a second party. In yet another embodiment each step of the method is performed by a different party.

In one embodiment, the unfertilized oocyte is a human oocyte. In another embodiment, the unfertilized oocyte is a non-human oocyte, e.g., the unfertilized oocyte is taken from a prize animal.

In one embodiment, the evaluation includes determining if the polar body has one or more chromosomes above or below the normal chromosome number. In one embodiment, when the evaluation indicates that the unfertilized oocyte associated with a polar body has one or more chromosomes above or below the normal number, the oocyte is discarded. In another embodiment, when the evaluation indicates that the unfertilized oocyte has one or more structural chromsomal abnormalities, the method further includes retrieving one or more oocytes from the donor. In another embodiment, when the evaluation indicates that the unfertilized oocyte associated with a polar body has a normal number of chromosomes, the oocyte is stored. In another embodiment, the method can further include using the stored oocyte with a normal number of chromosomes in a fertility or reproductive treatment, e.g. *in vitro* fertilization.

In one embodiment, the evaluation of the polar body includes the following steps: (a) providing one or more probes capable of hybridizing with a chromosome (or chromosomes) and/or a specific site on a chromosome (or chromosomes); (b) exposing the probe or probes to the chromosomes from the first polar body under conditions that allow the hybridization of the probe or probes to the target chromosome or chromosomes; and (c) detecting the probe or probes to thereby detect the chromosome and/or the site on the chromosome. The probe or probes can hybridize, e.g., to one or more of the following chromosomes or specific sites of these chromosomes: 13, 18, 16, 21, 22 or the X. In one embodiment, a probe or probes hybridized to one or more chromosomes can be analyzed by direct or indirect visualization methods.

In another embodiment, the evaluation of the polar body includes: (a) providing at least one nucleotide primer which is capable of hybridizing , e.g., under stringent conditions, to a chromsome or portion of a chromosome; (b) contacting the primer with chromosomal material from the first polar body under that allow the hybridization of the primer to the target chromosome, e.g., stringent conditions; (c) adding an elongating mixture that includes a nucleotide polymerase and a nucleotide mixture that includes at least one detectable nucleotide, under conditions that allow for elongation of a hybridized primer; and (d) analyzing the chromosomal material for elongated primers hybridized to the chromosomal material. The detectable nucleotide can be, e.g., a conjugated, modified and/or labeled nucleotide. The elongated primers on the chromosomal material can be, e.g., analyzed by direct or indirect

visualization of the elongated primer. In one embodiment, one or more primers hybridize to one or more of chromosomes 1 to 22, e.g., one or more of chromosomes 13, 16, 18, 21, 22, or X.

In one embodiment, the evaluation includes determining if the polar body has one or more chromosomes above or below the normal chromosome number and the absence or presence of more than one copy of the chromosome is indicative that the oocyte is unlikely to give rise to a healthy embryo. In one embodiment, the evaluation includes determining one or more of: the absence or presence of more than one copy of chromosome 21 in the polar body, wherein the absence of chromosome 21 is indicative of a risk for Down's Syndrome; the absence or presence of more than one chromosome 13 in the polar body, wherein the absence of chromosome 13 is indicative of a risk for Patau syndrome; the absence or presence of more than one copy of chromosome 18 in the polar body, wherein the absence of chromosome 18 is indicative of a risk for Edwards syndrome; the absence or presence of more than one chromosome 16 in the polar body, which is indicative of a risk for abnormal fetal development and/or miscarriage; the absence or presence of more than one chromosome 22 in the polar body, which is indicative of a risk for abnormal fetal development and/or miscarriage; the absence or presence of more than one chromosome 5 in the polar body, wherein the presence of more than one chromosome 5 is indicative of a risk for Cri du Chat; the absence or presence of more than one X chromosome in the polar body, wherein the absence of the X chromosome is indicative of a risk for Klinefelter's syndrome or Triple X syndrome and the presence of more than one of chromosome X is indicative of a risk for Turner's syndrome.

In another embodiment, the evaluation of the polar body includes determining if the polar body has one or more structural chromosomal abnormalities. In one embodiment, when the evaluation indicates that the unfertilized oocyte associated with a polar body has one or more structural chromosomal abnormality, the oocyte is discarded. In another embodiment, when the evaluation indicates that the unfertilized oocyte has one or more structural chromsomal abnormalities, the method further includes retrieving one or more oocytes from the donor. In another embodiment, when the evaluation indicates that the unfertilized oocyte associated with a polar body lacks one or more a structural chromosomal abnormality, the oocyte is stored. In another embodiment, the method can further include using the stored oocyte that lacks one or

more structural chromosomal abnormality in a fertility or reproductive treatment, e.g. *in vitro* fertilization.

In one embodiment, the evaluation for one or more structural abnormalities includes the following steps: (a) providing one or more probes capable of hybridizing with at least a portion of a chromosome (or chromosomes); (b) exposing the probe or probes to the chromosomes from the first polar body under conditions that allow the hybridization of the probe or probes to at least a portion of the target chromosome or chromosomes; and (c) detecting the probe or probes to thereby detecting at least a portion of the chromosome or chromosomes. The probe or probes can hybridize, e.g., to one or more of chromsomes 1 to 22 and the X chromosome, e.g., one or more of chromosomes 13, 14, 15, 21 or 22, or specific sites on the chromsome. In one embodiment, a probe or probes hybridized to one or more chromosomes can be analyzed by direct or indirect visualization methods.

In one embodiment, the evaluation for one or more structural chromosomal abnormality includes: (a) providing at least one nucleotide primer which is capable of hybridizing, e.g., under stringent conditions, to at least a portion of a chromosome; (b) contacting the primer with chromosomal material from a first polar body under conditions that allow the hybridization of the primer to its target, e.g., stringent conditions; (c) adding an elongating mixture that includes a nucleotide polymerase and a nucleotide mixture comprising at least one detectable nucleotide, under conditions that allow elongation of a hybridized primer; and (d) analyzing the chromosomal material for elongated primers hybridized to the chromosomal material. The detectable nucleotide can be, e.g., a conjugated, modified and/or labeled nucleotide. The elongated primers on the chromosomal material can be, e.g., analyzed by direct or indirect visualization of the elongated primer. In some embodiments, the chromosomal material is analyzed for one or more translocations. In one embodiment, the chromosomal material is analyzed for a translocation at one or more of chromosomes 13, 14, 15, 21 or 22. In some embodiments, the presence of a translocation at one or more of chromosomes 13, 14, 15, 21 or 22 is indicative of a risk for Robertsonian translocations. In some embodiment, the presence of a translocation, e.g., at one or more of chromosomes 13, 14, 15, 21 or 22, can result in the stored associated oocyte being discarded. In another embodiment, when the evaluation indicates the presence of one or more translocations, e.g. a translocation at one or more of chromosomes 13,

14, 15, 21 or 22, the method further includes retrieving one or more oocytes from the donor. In yet another embodiment, the evaluation indicates that the polar body lacks one or more translocations, e.g., lacks a translocation at one or more of chromosomes 13, 14, 15, 21 or 22, and the associated oocyte is stored. In another embodiment, the chromosomal material of the polar body is analyzed for one or more reciprocal translocation. In one embodiment, where one or more reciprocal translocation is present, the associated oocyte is discarded. In another embodiment, when the evaluation indicates the presence of one or more reciprocal translocations, the method further includes retrieving one or more oocytes from the donor. In another embodiment, when the analysis indicates that the polar body lacks one or more reciprocal translocations, the associated oocyte is stored.

In one embodiment, the evaluation of the first polar body includes determining a risk for one or more genetic disorders, e.g. one or more single-gene disorders. In one embodiment, the evaluation includes determining the absence or presence of one or more mutations (e.g., insertions, deletions or substitutions) in a gene or genes, e.g., a gene or genes associated with a disorder.

In some embodiments, the evaluation includes amplifying all or a portion of one or more genes of interest from the first polar body. The gene or portion of a gene of interest can be examined, e.g., for the absence or presence of one or more of the following: an insertion, deletion or substitution in the gene (or genes) of interest. In some embodiments, the gene or portion of a gene examined can be selected from one or more of the following: a gene encoding CFTR, a gene encoding dystrophin, a Beta Thalassemia gene, a gene encoding Factor VIII, a gene encoding Factor IX, Tay-Sachs gene, a survival motor neuron (SMN) gene, and a HD gene. In another embodiment, the first polar body can be evaluated for a genotype associated with one or more of the following genetic disorders: Adenoleukodystrophy, Amyotrophic Lateral Sclereosis (ALS), Becker Muscular Dystrophy, Beta Thalassemia, Cerebellar Ataxia, Charcot-Marie-Tooth Disease, Chondrodysplasia Aganglionic Megacolon, Conradi-Hunnerman Syndrome, Cystic Fibrosis, Duchenne Muscular Dystrophy, Hemophilia A or B, Huntington's Disease, Fragile X Syndrome, Glycogen Storage Disease, Hirschsprung Disease, Icthyosis, Lesch Nyhan, Myopathies, Polycystic Ovary Syndrome, Restenosis Pigmentosa, Sickle ell Anemia, Tay-Sachs Disease, and Von Willebran Disease.

In one embodiment, the evaluation of the first polar body includes (a) evaluating the presence or absence of one or more mutation, e.g., an insertion, deletion or substitution, in the gene or genes of interest from the polar body; and (b) comparing the evaluation of the gene or genes of interest from the polar body to both of the genes or portions of the genes from diploid maternal (i.e., oocyte donor) genetic material as an indication of what genes or regions of genes are present in the oocyte. In one embodiment, the oocyte donor is heterozygous carrier of a mutation or mutations in a gene or genes, e.g., a gene or genes associated with genetic disorder. In this embodiment, it is likely that the gene or genes of interest in the oocyte are the alternative gene (or genes) of interest from the maternal genetic material as is present in the polar body.

In another embodiment, evaluating the presence or absence of one or more mutation, e.g., one or more insertion, deletion or substitution, of the gene or genes of interest can be accomplished by amplifying genetic material, e.g., by Polymerase Chain Reaction (PCR).

In another embodiment, the evaluation includes contacting genetic material from the polar body with a probe (or probes) specific for a particular form of a gene, under conditions that allow the probe (or probes) specific to the particular form of a gene to hybridize with its target; e.g., a probe (or probes) which hybridize to one form of the gene, e.g., a mutated form of the gene but does not hybridize to a different form of the gene, e.g., a non-mutated form of the gene.

In one embodiment, the evaluation of the first polar body further includes storing or discarding the oocyte based on the absence or presence of one or more insertion, deletion or substitution of a gene or genes of interest. In one embodiment, where the oocyte donor is a heterozygous carrier of a genetic disorder, and the first polar body is found to lack one or more insertions, deletions, or substitutions associated with the genetic disorder, the associated oocyte is discarded. In another embodiment, where the evaluation indicates that the oocyte donor is a heterozygous carrier of a genetic disorder, and the first polar body is found to lack one or more insertions, deletions, or substitutions associated with the genetic disorder, the method further includes retrieving one or more oocytes from the donor. In another embodiment, if the oocyte donor is heterozygous carrier of a genetic disorder, and the first polar body is found to have one or more insertions, deletions, or substitutions associated with the genetic disorder, the associated oocyte is stored.

In another embodiment, the evaluation includes analyzing the morphology of the first polar body, e.g. according to one or more of the following morphological criteria: shape, surface smoothness, fragmentation, and size of the perivitalline space. In one embodiment, if the first polar body is determined to have one or more morphological features associated with poor embryo quality and/or poor embryo viability, the associated oocyte is discarded. In another embodiment, the evaluation indicates that the first polar body has one or more morphological features associated with poor embyo quality and/or poor embryo viability, and the method further includes retrieving one or more oocytes from the donor. In another embodiment, if the first polar body is determined to have one or more morphological feature associated with good embryo quality and/or viability the associated oocyte is stored, e.g., for use in a fertility or reproductive treatment.

In one embodiment, the evaluation indicates that the oocyte is suitable for storage and the method further includes treating the unfertilized oocyte to cause it to enter a dormant state prior to storage. In another embodiment, the evaluation indicates that the unfertilized oocyte is suitable for storage, and the method further includes storing the unfertilized oocyte, e.g., by freezing, drying or both, e.g., by plunge freezing, vacuum drying, air drying, freezer drying or combinations thereof. In one embodiment, the unfertilized oocyte is stored by freezing and the unfertilized oocyte is frozen to a cryogenic temperature. In another embodiment, the unfertilized oocyte is stored by freezing and the oocyte is cooled at a rate between about 0.3°C and 6°C per minute to a final temperature that is at least about -50° C. In another embodiment, the unfertilized oocyte is stored by freezing and the oocyte is cooled at a rate between 0.3°C and 3° C per minute to a final temperature that is between -50°C and -10° C. In another embodiment, the unfertilized oocyte is stored by drying and the oocyte is dried to a level sufficient to permit dry storage. In another embodiment, the evaluation indicates that the unfertilized oocyte is suitable for storage and the method further includes freeze drying the oocyte. In another embodiment, the evaluation indicates that the unfertilized oocyte is suitable for storage and the method further includes vacuum and/or convective drying the oocyte. In another embodiment, the evaluation indicates that the unfertilized oocyte is suitable for storage and the method further includes introducing prior to storage, e.g. by microinjection into the oocyte, e.g., microinjection into the cytoplasm of the unfertilized oocyte, a protective agent, e.g., a protective agent with one or more

of the following properties: (i) the agent includes a sugar or combination of sugars; (ii) the agent is substantially non-permeating; and (iii) it maintains the viability of the oocyte such that it can be stored in a temporarily dormant state and restored to an active state. In another embodiment, the method further includes contacting the oocyte to be stored with an extracellular protective agent that is substantially non-permeating with respect to mammalian cell membranes and that stabilizes the cell membrane of the oocyte. The protective agent includes, e.g., at least one sugar, e.g., a sugar selected from the group consisting of sucrose, trehalose, fructose, dextran, and raffinose. In another embodiment, the protective agent includes at least one sugar selected from the group consisting of: glucose, sorbitol, mannitol, lactose, maltose, and stachyose. In other embodiments, the protective agent includes at least one sugar with a glass transition temperature greater than -50° C and/or at least one sugar with a glass transition temperature greater than -30° C. In another embodiment, the protective agent includes at least one sugar with one of following properties: a molecular weight greater than 120 daltons; a glass transition temperature greater than -30° C and a molecular weight greater than 120 daltons. In another embodiment, the protective agent includes a glycolipid or a glycoprotein that includes at least one sugar moiety derived from a sugar with a glass transition temperature greater than -50° C. In another embodiment, a protective agent that includes a sugar is introduced into the oocyte and the cytoplasmic concentration of sugar is less than or equal to about 1.0 M following introduction of the sugar to the oocyte cytoplasm, but prior to causing the oocyte to enter a dormant state. In an alternate embodiment, a protective agent that includes sugar is introduced into the oocyte and the cytoplasmic concentration of sugar is less than or equal to about 0.2 M following introduction of the sugar to the oocyte cytoplasm, but prior to causing the oocyte to enter a dormant state. In another embodiment, the evaluation indicates that the oocyte is suitable for storage and the method further includes contacting the oocyte with an extracellular protective agent that includes a sugar. In another embodiment, the evaluation indicates that the oocyte is suitable for storage and the method further includes maintaining the oocyte in a liquid medium, e.g., with an extracellular concentration of sugar that is less than or equal to either about 1.0 M, or about 0.2 M, following dilution into the liquid medium. In another embodiment, the evaluation indicates that the oocyte is suitable for storage and the method further includes maintaining the oocyte on a solid medium, e.g., with an extracellular concentration of sugar that is less than or equal to either about 1.0 M, or to about 0.2 M. In yet other embodiments, a penetrating cryoprotectant

mixture is added to the protective agent, e.g., a protective agent described herein. In another embodiment, the method further includes introducing, e.g. by microinjectioninto the oocyte, e.g., microinjection into the cytoplasm of the oocyte, a protective agent and treating the unfertilized oocyte to cause it to enter a dormant state prior to storing the unfertilized oocyte.

In other embodiments, the method further includes treating the unfertilized oocyte that has been made dormant, e.g., as described herein, to restore the unfertilized oocyte to an active state. In one embodiment, the treatment to restore the unfertilized oocyte to an active state includes thawing the oocyte, rehydrating the oocyte or both. In one embodiment, the method further includes culturing the oocyte in a hypertonic medium having an osmolarity greater than 300 mosm to restore the oocyte to an active state.

In another embodiment, the method further includes entering into a database the results of the evaluation of the suitability of the unfertilized oocyte. In another embodiment, the method further includes correlating the results entered into the database with the location of the associated unfertilized oocyte in storage. In another embodiment, the method further includes entering into a database the code or value indicating the suitability of the unfertilized oocyte for storage and entering a code, value or location that correlates the suitability code or suitability value with the location of the unfertilized oocyte in storage. In another embodiment, the method further includes retrieving from storage an oocyte suitable for use in a fertility or reproductive treatment. This can include: (a) accessing a database described herein and (b) retrieving the oocyte from storage. In a related embodiment, the step (a) is performed by one party, and step (b) is performed by second party. In another related embodiment, both steps (a) and (b) are performed by the same party.

In one embodiment, the method includes providing an evalation of the first polar body from a second unfertilized oocyte, e.g., a second unfertilized oocyte from the same donor, that indicates the desirability of storing the second unfertilized oocyte; and storing or discarding the second oocyte based upon the results provided in the analysis of the polar body. The method can further include repeating the method on a third, fourth, fifth, etc. oocyte, e.g., from the same or a different donor.

In another aspect, the invention features a method of determining the suitability of at least two unfertilized oocyte for storage. The method includes the steps of: (a) providing at least two first polar bodies, each associated with a different unfertilized oocyte; (b) evaluating at least two of these first polar bodies to determine the desirability of storing the associated unfertilized oocyte; and (c) storing or discarding the unfertilized oocytes associated with each of the evaluated polar body based upon the evaluation of the first polar body.

In one embodiment, the unfertilized oocyte is a human oocyte. In another embodiment, the unfertilized oocyte is a non-human oocyte, e.g., the unfertilized oocyte is taken from a prize animal.

In one embodiment, all steps of the method are performed by one party. In another embodiment, steps (a) and (c) are performed by a first party and step (b) is performed by a second party. In another embodiment, steps (a) and (b) are performed by a first party and step (c) is performed by a second party. In yet another embodiment, step (a) is performed by a first party and steps (b) and (c) are performed by a second party. In yet another embodiment, each step of the method is performed by a different party.

In one embodiment, at least three or more (e.g., up to 10,15 or 20 oocytes from a single donor) first polar bodies are provided, each first polar body associated with a different unfertilized oocyte. In another embodiment, the method includes the steps of (b) evaluating all or some subset of the first polar bodies, each associated with a different unfertilized oocyte, in order to determine the desirability of storing each unfertilized oocyte that is associated with an evaluated first polar body; (c) comparing the evaluations; and (d) storing all, some, one or none of the unfertilized oocytes based upon the comparison of the evaluations of the associated first polar bodies. In one embodiment, all steps of the method are performed by one party. In another embodiment, steps (c) and (d) are performed by a first party and step (b) is performed by a second party. In another embodiment, steps (b) and (c) are performed by a first party and step (d) is performed by a second party. In another embodiment, steps (b) and (d) are performed by a first party and step (c) is performed by a second party. In another embodiment step (d) is performed by a party other than the one who performs any of the other steps. In yet another embodiment, each step of the method is performed by a different party.

In another embodiment, one or more associated unfertilized oocyte is discarded based on the comparison of the evaluations of one or more associated first polar bodies.

In another embodiment, one or more unfertilized oocytes is determined suitable for storing based, e.g., upon the coimparison of the evaulations of one or more associated first polar bodies, is stored in a manner that is appropriate for later use of the oocyte in a fertility or reproductive treatment, e.g. the unfertilized oocyte is stored in a manner suitable for later use in *in vitro* fertilization.

In one embodiment, at least one unfertilized oocyte that is determined suitable for storage, is stored by freezing the unfertilized oocyte, e.g., by freezing the oocyte as described herein. In another embodiment, at least one unfertilized oocyte is treated to cause it to enter a dormant state prior to storing the unfertilized oocyte, e.g., by a method described herein.

In another embodiment, the method further includes entering into a database the results of the evaluation of the suitability of one or more of the unfertilized oocytes. In one embodiment, the method further includes correlating the results with the location of the associated unfertilized oocyte. In another embodiment, at least two oocytes (or more) are determined to be suitable for storage and the method further includes entering into a database a code or value indicating the suitability of the unfertilized oocyte based upon the comparison of the evaluations of the associated polar bodies and providing a means for correlating the code or value with the unfertilized oocyte and/or the location of the unfertilized oocyte.

In another embodiment, the method further includes retrieving from storage one or more oocyte, e.g., for use in a fertility or reproductive treatment. The method can include: (a) accessing a database containing the results of an evaluation of one or more first polar body that indicating the suitability of an associated unfertilized oocyte, e.g., as compared to one or more of the other stored oocytes, or accessing a database that includes a value or code indicating the suitability of a stored oocyte, e.g., as compared to one or more of the other stored oocytes and (b) retrieving the oocyte from storage, e.g., for use in a fertility or reproductive treatment. In a related embodiment, step (a), accessing a database, is performed by one party, and step (b), retrieving the oocyte from storage is performed by second party. In another embodiment, both steps (a) and (b) are performed by the same party.

In one embodiment, the evaluation includes determining if one or more of the polar bodies has one or more chromosomes above or below the normal chromosome number, e.g., by a method described herein. In another embodiment, if an unfertilized oocyte associated with a polar body is determined to have one or more chromosome above or below the normal number, the oocyte is discarded. In one embodiment, all, some or one of the associated oocytes is determined to have one or more chromosome above or below the normal number, and the method further includes retrieving one or more oocytes from the donor. In another embodiment, all, some or one of the associated oocytes is determined to have the normal number of chromosomes and the oocytes having the normal number of chromosomes are stored. In another embodiment, the method further includes using one or more of the stored oocytes having a normal number of chromosomes in a fertility or reproductive treatment, e.g. *in vitro* fertilization. An evaluation of the polar body to determine if one or more of the oocytes has one or more chromosomes above or below the normal number, e.g., one or more of the chromosomes described herein, can be performed, e.g., by methods described herein.

In one embodiment, the evaluation of the polar bodies includes determining if one or more of the polar bodies has one or more structural chromosomal abnormality, e.g., by a method described herein. In one embodiment, if an unfertilized oocyte associated with a polar body is determined to have one or more structural chromosomal abnormality, the oocyte is discarded. In another embodiment, all, some or one of the associated oocytes is determined to have one or more structural chromosomal abnormality, and the method further includes retrieving one or more oocytes from the donor. In another embodiment, all, some or one of the associated oocytes is determined to lack one or more structural chromosomal abnormality and the oocytes lacking one or more structural chromosomal abnormality is stored. In another embodiment, the method further includes using one or more of the stored oocytes lacking one or more structural chromosomal abnormality in a fertility or reproductive treatment, e.g. *in vitro* fertilization. An evaluation of the polar body to determine if one or more of the oocytes has one or more structural chromosomal abnormality, e.g., one or more structural chromosomal abnormality described herein, can be performed, e.g., by methods described herein.

In one embodiment, the evaluation of the polar bodies includes determining if one or more of the associated oocytes is at risk for carrying one or more genetic disorders, e.g. one or

more single-gene disorders. In one embodiment, the evaluation includes determining the absence or presence of one or more mutations (e.g., insertions, deletions or substitutions) in a gene or genes, e.g., a gene or genes associated with a disorder, in the polar bodies, e.g., by a method described herein. In one embodiment, all some or one of the unfertilized oocyte associated with the polar bodies is determined to have one or more mutation, e.g., a mutation described herein, and the oocyte is discarded. In another embodiment, all, some or one of the associated oocytes is determined to have one or more mutation, e.g., a mutation described herein, and the method further includes retrieving one or more oocytes from the donor. In another embodiment, all, some or one of the associated oocytes is determined to lack one or more mutations, e.g., a mutation described herein, and the oocytes lacking the mutation or mutations is stored. In another embodiment, the method further includes using one or more of the stored oocytes lacking one or more mutation, e.g., a mutation described herein, in a fertility or reproductive treatment, e.g. *in vitro* fertilization. An evaluation of the polar body to determine if one or more of the oocytes has one or more mutations in a gene or genes, e.g., one or more mutations in a gene or genes associated with a disorder as described herein, can be performed, e.g., by methods described herein.

In another embodiment, the evaluation includes analyzing the morphology of the first polar bodies, e.g. according to one or more of the following morphological criteria: shape, surface smoothness, fragmentation, and size of the perivitalline space. In one embodiment, if all, some or one of the first polar bodies is determined to have one or more morphological feature associated with poor embryo quality and/or poor embryo viability, the associated oocyte is discarded. In another embodiment, if all, some or one of the first polar bodies is determined to have one or more morphological feature associated with good embryo quality and/or viability the associated oocyte is stored, e.g., for use in a fertility or reproductive treatment.

In another aspect, the invention features a method of selecting at least one unfertilized oocyte for use in fertility or reproductive treatment, and the method includes the steps of: (a) providing an analysis of the first polar body from an unfertilized oocyte which indicates the desirability of storing the unfertilized oocyte; (b) storing the unfertilized oocyte based upon the results obtained from the analysis or evaluation of the polar body; and (c) retrieving the

unfertilized oocyte from storage for use in a fertility or reproductive treatment, e.g., *in vitro* fertilization, thereby selecting an unfertilized oocyte for use in fertility or reproductive treatment.

In one embodiment, the unfertilized oocyte is a human oocyte. In another embodiment, the unfertilized oocyte is a non-human oocyte, e.g., the unfertilized oocyte is taken from a prize animal.

In one embodiment, all steps of the method are performed by one party. In another embodiment, steps (a) and (c) are performed by a first party and step (b) is performed by a second party. In another embodiment, steps (a) and (b) are performed by a first party and step (c) is performed by a second party. In yet another embodiment, step (a) is performed by a first party and steps (b) and (c) are performed by a second party. In yet another embodiment each step of the method is performed by a different party.

In one embodiment, the analysis includes a determination of whether the polar body has one or more chromosomes above or below the normal chromosome number. In one embodiment, based upon the analysis that the polar body that the unfertilized oocyte associated with a polar body has a normal number of chromosomes, the unfertilized oocyte is stored.

In one embodiment, the analysis indicates the presence of one of chromosomes, e.g. one of chromosomes 1-22, in the polar body thereby indicating that the oocyte is can give rise to a healthy embryo. In one embodiment, the analysis indicates one or more of: the presence one chromosome 21 in the oocyte; the presence of one chromosome 13 in the oocyte; the presence of one chromosome 18 in the oocyte; the presence of one chromosome 16 in the oocyte; the presence of one chromosome 22 in the oocyte; the presence of one chromosome 5; the presence of one X chromosome in the oocyte.

In another embodiment, the analysis of a polar body includes a determination of whether the polar body has one or more structural chromosomal abnormalities. In one embodiment, based upon the analysis that one or more structural abnormality is absent, the unfertilized oocyte is stored.

In some embodiments, the analysis indicates that one or more translocation is absent from the chromosomal material of the polar body. In some embodiments, the analysis indicates the absence of a translocation at one or more of chromosomes: 13, 14, 15, 21 or 22 of the polar body. In another embodiment, the analysis indicates that a reciprocal translocation is absent from the chromosomal material of the polar body.

In one embodiment, the analysis of the first polar body includes a determination of whether there is a risk that the oocyte may carry one or more genetic disorders, e.g. one or more single-gene disorders. In one embodiment, the analysis indicates the absence or presence of one or more mutations (e.g., insertion, deletion or substitution) in a gene or genes, e.g., a gene or genes associated with a genetic disorder, e.g., genetic disorders described herein. In some embodiments the analysis indicates the absence of a mutation or mutations in one or more genes associated with a disorder, e.g. in one or more genes described herein. In one embodiment, where the analysis indicates that the oocyte donor is heterozygous carrier of a genetic disorder, and the first polar body is found to lack one or more insertions, deletions, or substitutions associated with the genetic disorder, the associated oocyte is stored.

In another embodiment, the analysis includes a determination regarding the morphology of the first polar body, e.g. according to one or more of the following morphological criteria: shape, surface smoothness, fragmentation, and size of the perivitalline space. In one embodiment, the analysis indicates that the first polar body has one or more morphological features associated with good embryo quality and/or viability.

In one embodiment, the analysis indicates that at least one unfertilized oocyte is determined suitable for storage, and that unfertilized oocyte is stored, e.g., by freezing or drying, e.g., by a method described herein. In another embodiment, the analysis indicates that at least one unfertilized oocyte is suitable for storage, and that unfertilized oocyte is treated to cause it to enter a dormant state prior to storing the oocyte, e.g., by a method described herein.

In another embodiment, the method includes entering into a database the results of the analysis of the suitability of one or more unfertilized oocyte. In another embodiment, the method further includes correlating the results with the location of the associated unfertilized oocyte, e.g. the location of the associated oocyte in storage. In another embodiment, the method

further includes entering into a database a code or value indicating the suitability of the unfertilized oocyte for use in a fertility or reproductive treatment and entering a code, value, or location that correlates the suitability code or value with the unfertilized oocyte, or the location of the unfertilized oocyte.

In another embodiment, the method further includes (a) accessing the database containing the analysis of the first polar body that indicates the suitability of the associated unfertilized oocyte for use in fertilization or reproductive treatment, or (a) accessing a database containing a value or code (generated by analyzing a first polar body) indicating the suitability of a stored oocyte for use in a fertilization or reproductive treatment; and (b) retrieving the oocyte from storage for use in the fertility or reproductive treatment. In a related embodiment, step (a), accessing the database, is performed by one party, and step (b), retrieving the oocyte from storage, is performed by second party. In another embodiment, both steps (a) and (b) are performed by the same party.

In another aspect, the invention features a method of selecting an unfertilized oocyte for use in a fertility or reproductive treatment. The method includes the steps of: (a) providing at least one unfertilized oocyte and a first polar body from that unfertilized oocyte; (b) storing the unfertilized oocyte and first polar body; and (c) evaluating the stored first polar body to determine the suitability of the unfertilized oocyte for use in a fertility or reproductive treatment, e.g., *in vitro* fertilization.

In one embodiment, the unfertilized oocyte is a human oocyte. In another embodiment, the unfertilized oocyte is a non-human oocyte, e.g., the unfertilized oocyte is taken from a prize animal.

In one embodiment, all steps of the method are performed by one party. In another embodiment, steps (a) and (c) are performed by a first party and step (b) is performed by a second party. In another embodiment, steps (a) and (b) are performed by a first party and step (c) is performed by a second party. In yet another embodiment, step (a) is performed by a first party and steps (b) and (c) are performed by a second party. In yet another embodiment each step of the method is performed by a different party.

In another embodiment, the stored polar body is evaluated at a later date from storage, e.g., the polar body is analyzed 1, 2, 3, 4, 5, 6, 10, 12, 15, 20, 25, 30, 40, 50 or more months after placing the polar body in storage.

In one embodiment, the unfertilized oocyte is stored by freezing, drying or both, e.g., by a method described herein. In another embodiment, the unfertilized oocyte is stored in a manner that is appropriate for later use of the oocyte in a fertility or reproductive treatment, e.g. the unfertilized oocyte is stored in a manner suitable for later use in *in vitro* fertilization.

In another embodiment, the method further includes treating the unfertilized oocyte to cause it to enter a dormant state prior to storing the oocyte.

In another embodiment, the method further includes restoring the unfertilized oocyte to an active state, e.g., by a method described herein.

In another embodiment, the method includes entering into a database the location of the stored unfertilized oocyte (or a code or value indentifying the stored unfertilized oocyte), and correlating the location, code or value for the oocyte with a location of the asscoiated first polar body (or a code or value indentifying the first polar body). In another embodiment, the method further includes entering the results of the evaluation (or a code or value which indicates the suitability of the oocyte from the evaluation) into the database.

In another embodiment, the method further includes retrieving an oocyte from storage for use in a fertility or reproductive treatment. This can include: (a) accessing a database that includes one or more of the location (code or value) of the oocyte, the location (code or value) which correlates the first polar body to the oocyte, and the results of an evaluation of a first polar body indicating the suitability of an associated unfertilized oocyte for use in fertilization or reproductive treatment, and (b) retrieving the oocyte from storage. In a related embodiment, step (a), accessing a database, is performed by one party, and step (b), retrieving the oocyte from storage is performed by second party. In another embodiment, both steps (a) and (b) are performed by the same party.

In one embodiment, the evaluation includes determining if at least one polar bodies has one or more chromosomes above or below the normal chromosome number, e.g., by a method described herein. In another embodiment, if an unfertilized oocyte associated with a polar body is determined to have one or more chromosome above or below the normal number, the stored oocyte is discarded. In one embodiment, if the stored oocytes is determined to have one or more chromosome above or below the normal number, and the method further includes retreiving one or more oocytes from the donor. In another embodiment, if the stored oocyte is determined to have the normal number of chromosomes and the stored oocytes is retrieved. In another embodiment, the method further includes using at least one of the stored oocytes having a normal number of chromosomes in a fertility or reproductive treatment, e.g. *in vitro* fertilization. An evaluation of the polar body to determine if one or more of the stored oocytes has one or more chromosomes above or below the normal number, e.g., one or more of the chromosomes described herein, can be performed, e.g., by methods described herein.

In one embodiment, the evaluation of the polar bodies includes determining if at least one of the polar bodies has one or more structural chromosomal abnormality, e.g., by a method described herein. In one embodiment, if the stored oocyte associated with a polar body is determined to have one or more structural chromosomal abnormality, the oocyte is discarded. In another embodiment, if the stored oocyte is determined to have one or more structural chromosomal abnormality, and the method further includes retreiving one or more oocytes from the donor. In another embodiment, if the stored oocyte is determined to lack one or more structural chromosomal abnormality, the stored oocyte is retrieved. In another embodiment, the method further includes using one or more of the stored oocytes lacking one or more structural chromsomal abnormality in a fertility or reproductive treatment, e.g. *in vitro* fertilization. An evaluation of the polar body to determine if one or more of the stored oocytes has one or more structural chromsomal abnormality, e.g., one or more structural chromsomal abnormality described herein, can be performed, e.g., by methods described herein.

In one embodiment, the evaluation of at least one polar body includes determining if the of the associated oocyte is at risk for carrying one or more genetic disorders, e.g. one or more single-gene disorders. In one embodiment, the evaluation includes determining the absence or presence of one or more mutations (e.g., insertions, deletions or substitutions) in a gene or genes,

e.g., a gene or genes associated with a disorder, in the polar body, e.g., by a method described herein. In one embodiment, if the stored oocyte associated with the polar body is determined to have one or more mutation, e.g., a mutation described herein, the oocyte is discarded. In another embodiment, if the stored oocyte is determined to have one or more mutation, e.g., a mutaton decribed herein, the method further includes retreiving one or more oocytes from the donor. In another embodiment, if the stored oocyte is determined to lack one or more mutations, e.g., a mutation described herein, the oocyte lacking the mutation or mutations is retrieved. In another embodiment, the method further includes using one or more of the stored oocytes lacking one or more mutation, e.g., a mutation descibed herein, in a fertility or reproductive treatment, e.g. *in vitro* fertilization. An evaluation of the polar body to determine if one or more of the oocytes has one or more mutations in a gene or genes, e.g., one or more mutations in a gene or genes associated with a disorder as described herein, can be performed, e.g., by methods described herein.

In another embodiment, the evaluation includes analyzing the morphology of at least one first polar body, e.g. according to one or more of the following morphological criteria: shape, surface smoothness, fragmentation, and size of the perivitalline space. In one embodiment, if the first polar body is determined to have one or more morphological feature associated with poor embryo quality and/or poor embryo viability, the stored oocyte is discarded. In another embodiment, if the first polar body is determined to have one or more morphological feature associated with good embryo quality and/or viability the stored oocyte is retrieved and, e.g., used in a fertility or reproductive treatment, e.g., *in vitro* fertilization.

In another aspect, the invention includes a method of scoring the suitability of an unfertilized oocyte for use in fertilization or reproductive treatment. The method includes: (a) providing at least one unfertilized oocyte and a polar body from the unfertilized oocyte; (b) storing the unfertilized oocyte; and (c) evaluating the polar body to determine the suitability of using the stored oocyte in reproductive treatment. The method can further include: providing an indication of quality of the stored oocyte based upon the evaluation.

In one embodiment, the unfertilized oocyte is a human oocyte. In another embodiment, the unfertilized oocyte is a non-human oocyte, e.g., the unfertilized oocyte is taken from a prize animal.

In one embodiment, all steps of the method are performed by one party. In another embodiment, steps (a) and (c) are performed by a first party and step (b) is performed by a second party. In another embodiment, steps (a) and (b) are performed by a first party and step (c) is performed by a second party. In yet another embodiment, step (a) is performed by a first party and steps (b) and (c) are performed by a second party. In yet another embodiment, each step of the method is performed by a different party.

In one embodiment, the method further includes: assigning a value or code to the stored oocyte which indicates the suitability of the unfertilized oocyte for use in fertilization or reproductive treatment. In one embodiment, the party who performs the step of assigning a value or code is the same as a party who performs at least one other step in the method. In another embodiment, the party who performs the step of assigning a value or code is different from a party who performs another step in the method.

In one embodiment, the polar body is evaluated to determine the suitability of the oocyte for use in *in vitro* fertilization.

In one embodiment, the unfertilized oocyte is stored by freezing, drying or both, e.g., by a method described herein. In another embodiment, the unfertilized oocyte is stored in a manner that is appropriate for later use of the oocyte in a fertility or reproductive treatment, e.g. the unfertilized oocyte is stored in a manner suitable for later use in in vitro fertilization.

In another embodiment, the method further includes treating the unfertilized oocyte to cause it to enter a dormant state prior to storing the oocyte.

In another embodiment, the method further includes, e.g., based upon the evaluation, restoring the unfertilized oocyte to an active state, e.g., by a method described herein.

In another embodiment, the method includes entering into a database the location of the stored unfertilized oocyte (or a code or value indentifying the stored unfertilized oocyte), and

entering the results of the evaluation (or a code or value which indicates the score of the oocyte from the evaluation, e.g., as compared to the score of other oocytes evaluated, e.g., by the same methods) into the database.

In another embodiment, the method further includes retrieving an oocyte from storage for use in a fertility or reproductive treatment. This can include: (a) accessing a database that includes the location (code or value) of the oocyte, and the results of the evaluation of a first polar body, and (b) retrieving the oocyte from storage. In a related embodiment, step (a), accessing a database, is performed by one party, and step (b), retrieving the oocyte from storage is performed by second party. In another embodiment, both steps (a) and (b) are performed by the same party.

In one embodiment, the evaluation includes determining if at least one polar body has one or more chromosomes above or below the normal chromosome number, e.g., by a method described herein. In another embodiment, if an unfertilized oocyte associated with a polar body is determined to have one or more chromosome above or below the normal number, the stored oocyte is discarded. In one embodiment, if the stored oocytes is determined to have one or more chromosome above or below the normal number, and the method further includes retreiving one or more oocytes from the donor. In another embodiment, if the stored oocyte is determined to have the normal number of chromosomes and the stored oocytes is retrieved. In another embodiment, the method further includes using at least one of the stored oocytes having a normal number of chromosomes in a fertility or reproductive treatment, e.g. *in vitro* fertilization. An evaluation of the polar body to determine if one or more of the stored oocytes has one or more chromosomes above or below the normal number, e.g., one or more of the chromosomes described herein, can be performed, e.g., by methods described herein.

In one embodiment, the evaluation of the polar bodies includes determining if at least one of the polar bodies has one or more structural chromosomal abnormality, e.g., by a method described herein. In one embodiment, if the stored oocyte associated with a polar body is determined to have one or more structural chromosomal abnormality, the stored oocyte is discarded. In another embodiment, if the stored oocyte is determined to have one or more structural chromosomal abnormality, and the method further includes retreiving one or more

oocytes from the donor. In another embodiment, if the stored oocyte is determined to lack one or more structural chromosomal abnormality, the stored oocyte is retrieved. In another embodiment, the method further includes using one or more of the stored oocytes lacking one or more structural chromsomal abnormality in a fertility or reproductive treatment, e.g. *in vitro* fertilization. An evaluation of the polar body to determine if one or more of the stored oocytes has one or more structural chromsomal abnormality, e.g., one or more structural chromsomal abnormality described herein, can be performed, e.g., by methods described herein.

In one embodiment, the evaluation of at least one polar body includes determining if the of the associated oocyte is at risk for carrying one or more genetic disorders, e.g. one or more single-gene disorders. In one embodiment, the evaluation includes determining the absence or presence of one or more mutations (e.g., insertions, deletions or substitutions) in a gene or genes, e.g., a gene or genes associated with a disorder, in the polar body, e.g., by a method described herein. In one embodiment, if the stored oocyte associated with the polar body is determined to have one or more mutation, e.g., a mutation described herein, the oocyte is discarded. In another embodiment, if the stored oocyte is determined to have one or more mutation, e.g., a mutaton decribed herein, the method further includes retreiving one or more oocytes from the donor. In another embodiment, if the stored oocyte is determined to lack one or more mutations, e.g., a mutation described herein, the oocyte lacking the mutation or mutations is retrieved. In another embodiment, the method further includes using one or more of the stored oocytes lacking one or more mutation, e.g., a mutation descibed herein, in a fertility or reproductive treatment, e.g. *in vitro* fertilization. An evaluation of the polar body to determine if one or more of the oocytes has one or more mutations in a gene or genes, e.g., one or more mutations in a gene or genes associated with a disorder as described herein, can be performed, e.g., by methods described herein.

In another embodiment, the evaluation includes analyzing the morphology of at least one first polar body, e.g. according to one or more of the following morphological criteria: shape, surface smoothness, fragmentation, and size of the perivitalline space. In one embodiment, if the first polar body is determined to have one or more morphological feature associated with poor embryo quality and/or poor embryo viability, the stored oocyte is discarded. In another embodiment, if the first polar body is determined to have one or more morphological feature

associated with good embryo quality and/or viability the stored oocyte is retrieved and, e.g., used in a fertility or reproductive treatment, e.g., *in vitro* fertilization.

In another aspect, the invention features a method of providing information regarding the suitability of an unfertilized oocyte for fertility or reproductive treatment. In one embodiment, the method includes: (a) providing a first polar body from an unfertilized oocyte; (b) amplifying all or part of the genome from the polar body; and (c) storing all or part of the genome from the polar body for later analysis of the suitability of the unfertilized oocyte for use in a fertility or reproductive treatment. In another embodiment, the method includes: (a) providing a first polar body from an unfertilized oocyte; (b) amplifying all or part of the genome from the polar body; and (c) evaluating one or more genes (or portions thereof) from the amplified genome to determine the suitability of the unfertilized oocyte for fertility or reproductive treatment. In one embodiment, all steps of the method are performed by one party. In another embodiment, steps (a) and (c) are performed by a first party and step (b) is performed by a second party. In another embodiment, steps (a) and (b) are performed by a first party and step (c) is performed by a second party. In another embodiment, step (a) is performed by a first party and steps (b) and (c) are performed by a second party. In yet another embodiment, each step of the method is performed by a different party.

In one embodiment, the unfertilized oocyte is a human oocyte. In another embodiment, the unfertilized oocyte is a non-human oocyte, e.g., the unfertilized oocyte is taken from a prize animal.

In one embodiment, the method further includes storing all or a portion of the amplified genome for later evaluation, e.g. the evaluation takes place 1, 2, 3, 4, 5, 6, or 9 mo, or 1, 2, 3, 4, 5, 6, 10 or more years after amplification.

In another embodiment, the method provides information regarding the suitability the oocyte for use *in vitro* fertilization.

In one embodiment, the method further includes storing the polar body prior to amplification, e.g., storing by a method described herein. In one embodiment, the polar body is evaluated, e.g., to determine if the polar body has one or more chromosomes above or below the

normal chromosome number; to determine if the polar body has one or more structural chromosomal abnormalities; and/or to determine the morphology of the first polar body, prior to storing the polar body. An evaluation of the polar body to determine if one or more of the associated oocytes has one or more chromosomes above or below the normal number, e.g., one or more of the chromosomes described herein, can be performed, e.g., by methods described herein. An evaluation of the polar body to determine if one or more of the stored oocytes has one or more structural chromosomal abnormality, e.g., one or more structural chromosomal abnormality described herein, can be performed, e.g., by methods described herein. An evaluation of the morphology of the first polar body, e.g., by one or more of the criteria described herein, can be performed, e.g., by a method described herein. In one embodiment, the polar body can be evaluated to determine one or more of: if the polar body has one or more chromosomes above or below the normal chromosome number; if the polar body has one or more structural chromosomal abnormalities; and/or the morphology of the first polar body, prior to storing the polar body, and the polar body can be stored, e.g., on a slide or other support used to make the evaluation.

In another embodiment, the method further includes storing the oocyte associated with the first polar body, e.g., storing the oocyte prior to one or more of: storing the polar body, amplifying all or a portion of the genome of the polar body, and evaluation of one or more genes (or portions thereof) from the amplified genome. In one embodiment, the unfertilized oocyte is stored by freezing, drying or both, e.g., by a method described herein. In another embodiment, the unfertilized oocyte is stored in a manner that is appropriate for later use of the oocyte in a fertility or reproductive treatment, e.g. the unfertilized oocyte is stored in a manner suitable for later use in in vitro fertilization. In another embodiment, the method further includes treating the unfertilized oocyte to cause it to enter a dormant state prior to storing the oocyte.

In another embodiment, the method includes: entering into a database one or more of: a location of the oocyte in storage (or code or value identifies the stored oocyte); the location of the polar body in storage (or a code or value that identifies the polar body, and preferably, correlates it to its associated oocyte); the results of an evaluation of the polar body, e.g., for chromosomal number, structural abnormalities, and/or morphology; the results of the evaluation of the amplified genomic material from the polar body. In one embodiment, the method can

further include correlating one or more of the results with the location of the associated unfertilized oocyte. In another embodiment, the method further includes retrieving an oocyte suitable for use in a fertility or reproductive treatment from storage. These steps include: (a) accessing a database described herein and (b) retrieving the oocyte from storage. In a related embodiment, step (a), accessing a database, is performed by one party, and step (b), retrieving the oocyte from storage, is performed by second party. In another embodiment, both steps (a) and (b) are performed by the same party.

In one embodiment, the evaluation of the amplified genome or portion of the genome of the first polar body includes determining a risk that the oocyte is a carrier for one or more genetic disorders, e.g. one or more single-gene disorders. For example, the amplified genome or portion of the genome can be examined for the absence or presence of one or more of the following: an insertion, deletion or substitution in the gene or genes of interest. In some embodiments, the gene or portion of a gene is selected from one or more of the following: a gene encoding CFTR, a gene encoding dystrophin, a Beta Thalassemia gene, a gene encoding Factor VIII, a gene encoding Factor IX, Tay-Sachs gene, a survival motor neuron (SMN) gene, and a HD gene. In another embodiment, the amplified genome or portion of the genome of the first polar body is evaluated for a genotype associated with one or more of the following genetic disorders: Adenoleukodystrophy, Amyotrophic Lateral Sclereosis (ALS), Becker Muscular Dystrophy, Beta Thalassemia, Cerebellar Ataxia, Charcot-Marie-Tooth Disease, Chondrodysplasia Aganglionic Megacolon, Conradi-Hunnerman Syndrome, Cystic Fibrosis, Duchenne Muscular Dystrophy, Hemophilia A or B, Huntington's Disease, Fragile X Syndrome, Glycogen Storage Disease, Hirschsprung Disease, Icthyosis, Lesch Nyhan, Myopathies, Polycystic Ovary Syndrome, Restenosis Pigmentosa, Sickle ell Anemia, Tay-Sachs Disease, and Von Willebran Disease. An evaluation of the amplified genome or portion of the genome can be performed, e.g., by a method described herein.

In another aspect, the invention features a method for providing information regarding an unfertilized oocyte. The method includes: (a) providing a first polar body from an unfertilized oocyte; (b) storing the polar body; and (c) retrieving the polar body at a later date, e.g. after 1, 2, 3, 4, 5, 6, 7, 9, 10, or 11 months or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 or 15 years or more, to evaluate

the first polar body and determine the suitability of the unfertilized oocyte for use in a fertility or reproductive treatment based upon that evaluation.

In another aspect, the invention features a method for providing information regarding an unfertilized oocyte. The method includes: (a) providing a polar body from an unfertilized oocyte; (b) treating the polar body to allow visualization of its chromosomes; e.g. fixing the polar body e.g. on a slide; (c) storing the treated polar body chromosomes; and (d) retrieving the chromosomes at a later date, e.g. after 1, 2, 3, 4, 5, 6, 7, 9, 10, or 11 months or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 or 15 yrs, to further evaluate the suitability of the unfertilized oocyte for use in a fertility or reproductive treatment.

Detailed Description of the Invention

The freezing of oocytes allows women who may, in the future, experience infertility due to aging or other factors such as, e.g., radiation or chemotherapy, to store their eggs and have them available for fertilization many years later. The invention provides methods of determining the suitability of an unfertilized egg for storage and future fertilization. The suitability of an oocyte for storage can be determined by obtaining the first polar body from an unfertilized oocyte. The phrase "suitability of an oocyte" refers to one or more characteristic of an oocyte that makes it more likely to give rise to a healthy embryo than an oocyte that does not have that characteristic. Such characteristics include, for example, 1) the presence of one copy of a particular chromosome; 2) the presence of a normal number of chromosomes, i.e., 23 chromosomes; 3) the absence of a translocation; 4) the absence of one or more mutations (e.g., insertion, deletion or substitution) in a gene or genes, e.g., the absence of one or more mutations in a gene or genes that is associated with a genetic disorder.

Obtaining Oocytes from a Subject

Various methods are known for inducing ovulation to obtain oocytes for storage and use in human *in vitro* fertilization procedures. Any method of inducing oocyte stimulation can be used to provide an oocyte. Some of the more widely used protocols are described below.

Pituitary down-regulation with GnRH agonist

Briefly, pituitary down-regulation involves daily injections of a GnRH agonist, Lupron®. The Lupron® is administered until "down-regulation" of pituitary GnRH receptors has occurred and pituitary FSH and LH release is reduced to a minimum. Generally, the GnRH agonist is administered for a period of about 10-15 days before down-regulation occurs. Down-regulation can be confirmed by the onset of menses and a serum estradiol level that is less than 50 pg/ml. One of the quicker ways to achieve down-regulation is to start the GnRH agonist in the mid-luteal phase. It can also be started in the early follicular phase with the onset of menses. After down-regulation has occurred, the dose of the GnRH agonist can be reduced and the ovulation induction can be initiated with a daily FSH injection.

Microdose-Lupron protocol

This protocol can be used for women who are poor responders or who have evidence of reduced ovarian reserve. Briefly, this protocol involves the administration of oral contraceptives for a period of 3 weeks. After the 3-week course of the oral contraceptives has been completed, then microdoses of Lupron® and FSH are administered twice daily. When Lupron® is administered in this fashion, it acts as a stimulatory agent by inducing the release of FSH and LH.

Pituitary suppression with GnRH antagonist

Another protocol utilizes a GnRH antagonist, which suppresses a LH surge. In contrast to the GnRH agonist, the pituitary suppression achieved by GnRH antagonist is fairly rapid. Briefly, for this protocol, gonadotropins are initiated on cycle day 2. When the lead follicle reaches a diameter of 14 mm, the GnRH antagonist is administered (with the gonadotropins) on a daily basis.

Monitoring ovarian stimulation

During the ovarian stimulation, a woman's response to treatment can be monitored with serum estradiol levels and the vaginal ultrasound examinations. A mature follicle as used herein refers to one that is at least 15-17 mm in diameter. Once this is achieved, the FSH and other

medications are discontinued. The woman can then take a single injection of hCG, which further matures the egg to allow them to become fertilized. If it is judged that the response is insufficient, the cycle is cancelled and the treatment plan is reassessed.

Egg retrieval

The following is a brief description of a protocol that can be used to retrieve the oocytes from a female. The egg retrieval can be performed under vaginal ultrasound guidance. After the vaginal ultrasound is placed in the vagina and the ovarian follicles are located, a needle can be directed through the back wall of the vagina and directly into the ovarian follicles. The fluid is aspirated and then examined by an embryologist to see if an egg has been retrieved. All follicles within both ovaries are aspirated. Once the eggs are retrieved, they are placed in culture plates with nutrient media and then placed in the incubators. The procedure is performed under a light anesthesia and takes less than 5-10 minutes to perform. Progesterone is started the evening after the egg retrieval. Other methods and techniques for retrieving an oocyte can also be used.

Methods of Obtaining the First Polar Body

Methods of obtaining a first polar body from an unfertilized oocyte are known. For example, the first polar body can be isolated from an oocyte by enzymatic or mechanical means. For enzymatic removal of a polar body, the oocyte can be treated with an agent such as trypsin or PBS. Using a stereomicroscope, it can be determined when the zona begins to disappear. Once this occurs, the polar body can be separated from the oocyte. Mechanical removal of a polar body can be carried out with a micromanipulator (Narishige, Tokyo, Japan). Methods of mechanically removing a first polar body from a human oocyte are described, e.g., in Verlinsky et al. (1990) Human Reprod. 5:826-829.

A polar body can be obtained after harvesting an oocyte. For example, the polar body can be obtained within 10, 15, 20, 25, 30, 45 minutes, 1 hour up to 2, 3, 4, or 5 hours after the oocyte has been harvested. Preferably, the polar body is obtained within about an hour after the oocyte is harvested.

Methods of Analyzing the First Polar Body

The methods of the invention can include analyzing the first polar body of an unfertilized oocyte to determine the suitability of the associated oocyte for storage. In addition, the methods of the invention can include analyzing a first polar body, e.g., a stored polar body, to determine the suitability of a stored oocyte for use in a reproductive treatment such as *in vitro* fertilization.

Various information can be provided from the first polar body to evaluate the suitability of the associated oocyte for storage and/or use in a reproductive treatment. For example, chromosomal material from the polar body can be analyzed to determine if more or less than the total normal chromosomal number (i.e., 23) is present in the polar body. In addition, chromosomal material from the polar body can be analyzed to determine if a particular chromosome or chromosomes are absent, present in one copy or present in more than one copy in the polar body. If the polar body has more than one copy of a chromosome, it indicates that the chromosome may be absent from the associated oocyte. If a polar body does not have the chromosome, it indicates that the associated oocyte may have more than one copy of the chromosome. Fertilization of such an oocyte can result in aneuploidy, i.e., an embryo having too many or too few chromosomes. Having an extra chromosome is referred to as "trisomy", missing a chromosome is referred to as "monosomy".

There can be several consequences that can result when an embryo is an aneuploid. For example, if an embryo is aneuploid for any one of the autosomal chromosomes (chromosomes 1 to 22), the embryo may not implant and/or may stop developing in utero. Examples of autosomal chromosomes that have been associated with an increased risk of abnormal implantation, abnormal development, embryo death and/or miscarriage include chromosome 16 and chromosome 22. In addition, some embryos that are aneuploids can develop to term but the resulting infant may have physical and/or mental problems. Examples of such chromosomes include chromosome 13, chromosome 18, chromosome 21 and the X chromosome. An additional copy of chromosome 13 has been associated with Patau syndrome; an additional copy of chromosome 21 has been associated with Down's syndrome; an additional copy of chromosome 18 has been associated with Edward's syndrome; an additional copy of the X

chromosome has been associated with Klinefelter's syndrome or Triple X syndrome; and the absence of chromosome X has been associated with Turner's syndrome.

In some aspects, the methods of the invention can be used to determine if there is a risk that an oocyte is aneuploid. Such a determination can allow a subject who wants to store an oocyte for future use to decide whether or not to store an oocyte. In addition, such a determination can allow a subject to decide whether to use an oocyte that has been stored for a reproductive treatment such as *in vitro* fertilization. It also gives a method of scoring the quality of two or more eggs to be stored and provides the subject with the option of harvesting more eggs to potentially store.

The methods of the invention can also be used to evaulate whether the oocyte has one or more structural chromosomal abnormality, by evaluating whether the first polar body has one or more chromosomes with a structural abnormality or abnormalities. "Structural chromosomal abnormalties" occur when there is a change in the structure or components of a chromosome. The total number of chromosomes in an unfertilized oocyte or a first polar body is usually normal (23 total per cell). Structural chromosome abnormalities occur when part of a chromosome is missing, a part of a chromosome is extra, or a part has switched places with another part. An example of a structural chromosomal abnormality is a translocation. Translocations occur when pieces of a chromosome are attached to the wrong chromosome.

Two common translocations are Robertsonian translocations and reciprocal translocations. Robertsonian translocations can typically occur between one or more of chromosomes 13, 14, 15, 21 and 22. Robertsonian translocation can either contain the correct amount of genetic material (be balanced) or contain an unbalanced amount of genetic material (unbalanced). If an egg contains an unbalanced amount of genetic material and fertilization occurs, the resulting embryo will have too many copies or parts of one chromosome and/or too few copies or parts of the other. This can result in too many or too few normal genes on a chromosome. An unbalanced state in an embryo may lead to embryo death, miscarriage, or the birth of an infant with mental and/or physical problems. Reciprocal translocations are the exchange of chromosomal material between the wrong chromosomes. If this exchange breaks a gene, the subject can have a genetic disease. If the amount of genetic material present is the

same as with normal individuals, the subject is balanced and normal. However, the sperm or eggs of these individuals can carry the reciprocal translocation chromosome and are at increased risk of producing an embryo with an abnormal amount of genetic material (be unbalanced). As with Robertsonian translocations, there is an increased risk of embryo death, miscarriage or the birth of an infant with mental and/or physical problems. By evaluating whether there is a risk that an oocyte carries a structural chromosomal abnormality, a decision can be made whether to store or discard the oocyte and/or whether to harvest additional oocytes.

Aneuploidy and structural chromosomal abnormalities can be evaluated by methods known in the art. For example, the first polar body can be fixed and a stain such as Leishman's stain can be applied such that the total number of chromosomes in the polar body can be visualized. See, e.g., Durban et al. (1998) Human Reprod. 13 (3):583-587. Aneuploidy and structural chromosomal abnormalities can also be detected using *in situ* hybridization methods (described, e.g., in Fan, Y. *et al.* (1990) *Proc. Natl. Acad. Sci. USA*, 87:6223-27).

Fluorescence *in situ* hybridization (FISH) of a probe or probes to a chromosomal spread can be used to determine if all or part of a chromosome is present in a polar body. Probes for chromosome mapping can be used individually to mark a single chromosome or a single site on that chromosome, or panels of probes can be used for marking multiple sites and/or multiple chromosomes. In some embodiments, a probe or a panel of probes can be used that detect one or more of chromosomes 13, 16, 18, 21, 22 and the X chromosome. Such probes can identify the absence or presence of these chromosomes in the polar body. In other embodiments, a probe or a panel of probes can be used that detects structural chromosomal abnormalities at one or more of chromosomes 13, 14, 15, 21 and 22. In addition, a panel of probes can be used, at least one of which detects the absence or presence of a chromosome and at least one which detects a structural chromosomal abnormality of a chromosome. A probe or probes which hybridizes to nucleic acids from the desired chromsome can be selected. As used herein, the term "hybridizes under low stringency, medium stringency, high stringency, or very high stringency conditions" describes conditions for hybridization and washing. Guidance for performing hybridization reactions can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6, which is incorporated by reference. Aqueous and nonaqueous methods are described in that reference and either can be used. Specific hybridization conditions referred to

herein are as follows: 1) low stringency hybridization conditions in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by two washes in 0.2X SSC, 0.1% SDS at least at 50°C (the temperature of the washes can be increased to 55°C for low stringency conditions); 2) medium stringency hybridization conditions in 6X SSC at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 60°C; 3) high stringency hybridization conditions in 6X SSC at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 65°C; and preferably 4) very high stringency hybridization conditions are 0.5M sodium phosphate, 7% SDS at 65°C, followed by one or more washes at 0.2X SSC, 1% SDS at 65°C. Very high stringency conditions (4) are the preferred conditions and the ones that should be used unless otherwise specified. Probes that identify each of the human chromosomes and probes that can detect chromosomal abnormalities in human chromosomes are commercially available.

If more than one probe is being used, each of the probes can be labeled with the same or a different label. If the same label is being used for each probe, the probe can stripped from the spread prior to contacting the spread with the next labelled probe. Examples of labels that can be used include SpectrumRed, SpectrumAqua, SpectrumBlue, SpectrumGreen and Spectrum Gold, all commercially available from Vysis Inc., Downers Grove, IL.

Primed in situ labeling (PRINS) can also be used to evaluate an oocyte for aneuploidy and/or structural chromosomal abnormalities. Briefly, a primer or primers can be used to mark a single chromosome or a single site on that chromosome or a panel of primers can be used to mark multiple sites and/or multiple chromosomes. Denatured chromosomes from the polar body are contated with a primer or primers selected to hybridize with a chromosome (or chromosomes) or a site or sites on a chromosome (or chromsomes). Primer extension can be carried out with the appropriate polymerase in the presence of labeled nucleotides. The nucleotides can be directly or indirectly labeled. The labeled site can then be visualized by detecting the label. Methods of performing direct- and indirect- PRINS are described, e.g., in Musio et al. (1998) Biochemica 2:29-30.

In some embodiments, when the polar body is evaluated as an indicator of aneuploidy and/or structural chromosomal abnormality using PRINS, a primer or a panel of primers can be used that detect one or more of chromosomes 13, 16, 18, 21, 22 and the X chromosome. Such

primers can identify the absence or presence of these chromosomes in the polar body. In other embodiments, a primer or a panel of primers can be used that detects structural chromosomal abnormalities at one or more of chromosomes 13, 14, 15, 21 and 22. In addition, a panel of primers can be used, at least one of which detects the absence or presence of a chromosome and at least one which detects a structural chromosomal abnormality of a chromosome.

The suitability of an oocyte for storage and/or use in a reproductive treatment can also be evaluated by screening a nucleic acid (or nucleic acids) obtained from the polar body to determine if the polar body carries a selected mutation (or mutations) in a gene or genes, e.g., a gene or genes known to be associated with a disorder. For example, the oocyte donor may be a heterozygous carrier of a mutation (or mutations) in a gene or genes associated with a genetic disorder. Thus, the donor carries two different alleles, one that has the mutation in the gene and one that does not. Once it has been determined that the mutation (or mutations) is absent or present in chromosomal material from the polar body, it can be assumed that the associated oocyte has the opposite allele of the donor genetic material than that of the polar body. Therefore, if the donor is heterozygous for a particular mutation (or mutations) in a gene and that mutation (or mutations) is present in the chromosomal material from the polar body, it is likely that the chromosomal material of the associated oocyte does not carry that mutation (or mutations). If the mutation or mutations are absent from the chromosomal material of the polar body, it is likely that the chromosomal material from the oocyte carries that mutation or mutations. Thus, the methods described herein can be used to detect genetic alterations in a selected gene or genes, e.g., a gene or genes known to be associated with a disorder, to thereby determining if the oocyte is at risk for carrying the mutation or mutations associated with the disorder. In preferred embodiments, the methods include detecting, from the nucleic acid sample obtained from the polar body, the presence or absence of a genetic alteration characterized by at least one of an alteration affecting the integrity of a gene encoding the selected gene. For example, such genetic alterations can be detected by ascertaining the existence of at least one of 1) a deletion of one or more nucleotides from a gene; 2) an addition of one or more nucleotides to a gene; 3) a substitution of one or more nucleotides of a gene, 4) a chromosomal rearrangement of a gene; 5) an inversion of one or more nucleotides of a gene, and 6) allelic loss of a gene.

Such methods can be used to screen for oocytes that may be carriers of a mutation (or mutations) in one or more genes that is associated with a disorder. The information gained by these methods can be used to select for storage (or use of such stored oocytes) only those oocytes considered unlikely to be a carrier of the mutated gene or genes associated with the specific genetic disorder for which testing is performed. Various disorders including: Spinal Musclar Atrophy (SMA) Type I, Huntington's disease, and cystic fibrosis, have been associated with specific mutations. For example, deletions in the survival motor neuron (SMN) gene have been identified in 98% of SMA type I cases. In addition, the presence of the deltaF508 mutation in the CTFR gene accounts for 75% of identified cystic fibrosis mutations.

Other genes which can be screened include: the gene encoding dystrophin, the Beta Thalassemia gene, the gene encoding Factor VIII, the gene encoding Factor IX, Tay-Sachs gene, and the HD gene. Examples of disorders for which the oocyte can be evaluated include but are not limited to: Adenoleukodystrophy, Amyotrophic Lateral Sclereosis (ALS), Becker Muscular Dystrophy, Beta Thalassemia, Cerebellar Ataxia, Charcot-Marie-Tooth Disease, Chondrodysplasia Aganglionic Megacolon, Conradi-Hunnerman Syndrome, Cystic Fibrosis, Duchenne Muscular Dystrophy, Hemophilia A or B, Huntington's Disease, Fragile X Syndrome, Glycogen Storage Disease, Hirschsprung Disease, Icthyosis, Lesch Nyhan, Myopathies, Polycystic Ovary Syndrome, Restenosis Pigmentosa, Sickle Cell Anemia, Tay-Sachs Disease, and Von Willebran Disease.

By evaluating whether there is a risk that an oocyte carries a mutation in a gene or genes associated with a particular disorder, a decision can be made whether to store or discard the oocyte and/or whether to harvest additional oocytes.

Genetic mutations in a gene or a portion of a gene can be determined by methods known in the art. For example, the actual base-by-base DNA sequence of selected portions of the polar body's genetic material can be determined by amplifying all or a portion of a gene from the DNA of the polar body and subsequently sequencing it. A set of primers suitable for use in a PCR can be used to amplify all or a portion of a gene from the polar body. The primers should be at least 5, 10, or 50 base pairs in length and less than 100, or less than 200, base pairs in length. The primers should be identical, or differs by one base from a sequence disclosed herein or from a

naturally occurring variant. In other embodiments, genetic mutations in a gene or portion of a gene can be detected by hybridizing a nucleic acid from the polar body with a probe or probes which hybridize the specified nucleic acid, e.g., a probe or probes that can specifically hybridize to a nucleic acid encoded by a specific allele of a selected gene. For example, a probe or probes can be used that can distinguish from a first mutant and a second non-mutant form of a gene. Primers for amplifying specific genes or portions of genes associated with various disorders are known, as are probes for detecting various mutations in such genes.

In yet other embodiments, the suitability of an oocyte can be evaluated by analyzing the morphology of the first polar body. For example, the morphology of the first polar body can be evaluated based upon one or more of the following characteristics: shape; surface smoothness; fragmentation; and size of the perivitalline space. The associated oocyte can be selected if the morphology of the first polar body is normal for at least one of these characteristics.

Storing the Oocyte and Polar Body

Methods for storing oocytes for use in a fertility or reproductive treatment are known. For example, drying and freezing methods may be used to store oocytes.

Methods of vacuum or air drying and for freeze drying proteins are well known. See Franks et al., "Materials Science and the Production of Shelf-Stable Biologicals," BioPharm, October 1991, p. 39; Shalaev et al., "Changes in the Physical State of Model Mixtures during Freezing and Drying: Impact on Product Quality," Cryobiol. 33, 14-26 (1996). Such protocols may be used to prepare oocyte suspensions for storage. In addition to air drying, other convective drying methods that may be used to remove water from cell suspensions include the convective flow of nitrogen or other gases, and preferably do not contain oxygen which may be deleterious to certain cells. One example of such drying methods includes: the evaporative vacuum drying of 20 microliters each into wells on 12 well plates and vacuum drying for 2 hours at ambient temperature. Other drying methods can be used, including drying the cells in vials. Cells prepared in this manner may be stored dry, and rehydrated by diluting in DMEM or any other suitable media.

Several methods for the freezing, also referred to as cryopreservation, of an oocyte are known. These include the use of penetrating cryoprotectants such as dimethyl sulfoxide (DMSO), gylcerol and or ethylene glycol at concentrations of about 1M to 2 M, followed by slow rate freezing, e.g., at about 0.3°C to 0.5°C/minute. Another method of freezing oocytes is known as vitrification (i.e. formation of glassy material without crystallization of ice). This procedure uses high concentrations of cryoprotectant mixtures (e.g., about 6M to 8M) followed by rapid cooling.

Additional methods include using a cryoprotectant that includes propanediol (PROH) and a sugar such as sucrose. For example, the following method can be used. Briefly, after egg collection, the egg or eggs are maintained in culture for at least 5 hours. Eggs are stripped in hyaluronidase, e.g., at any point during the pre-incubation period. The eggs are then placed in a warm modified HTF medium and placed at room temperature for about 10 to 20 minutes. Preferably, the oocytes are maintained at room temperature until they reach a temperature of about 20°C to 25°C, preferably about 22°C. Oocytes are exposed to 1.5M 1,2-propanediol (propylene glycol) (PROH) in modified HTF for about 20 minutes and then are placed into 1.5M PROH and 0.2M sucrose for an addition 10 to 20 minutes. A cryovial is rinsed with PROH and sucrose medium and then filled with 0.3 ml of this medium in order to receive the oocytes. Oocytes are then frozen in the following manner:- 22°C down to -5.0°C at a rate of 2.0 °C/minute, then held 15 minutes at -5.0° C, "seeded" after 5 minutes, ensuring that the "seed" has been established. Afterwards, the oocyte is cooled further at 0.3°C/minute to - 38°C and then plunge into liquid nitrogen for storage. For thawing, cryovial is placed at room temp for 1 minute, then placed in 30°C water bath until ice crystals are gone. Contents are removed into a room temp droplet containing 1.5M PROH and 0.3M sucrose in modified HTF with 10% HSA, then placed into subsequent PROH dilutions for 8 minutes at each of 1.0M, 0.75M, 0.5M, 0.25M, 0.0M, all with 0.3M sucrose. Dilute slowly to the final 0.3M sucrose drop with modified HTF and 10% HSA, wash the eggs through 4 to 5 drops modified HTF, then 6 to 8 drops plain HTF and 10% HSA. The eggs can then be placed in the incubator.

Another method for freezing oocytes that utilizes PROH and a sugar such as sucrose is as follows: oocytes are pre-equilibrated in 1.5M 1, 2 PROH, and 1.5M PROH containing 0.2M sucrose for 5 minutes each at 37° C, the medium may include other cryoprotectants. Oocytes are

cooled using a controlled cooling rate to -70° C, at a rate of about -20° C/minute or -30° C/minute before seeding and further cooling. After thawing, cryoprotectants are removed by stepwise dilution. Oocytes that survive the freezing/thawing procedures are cultured in HTF with 10% serum for 2 hours to further assess their viability.

In some embodiments, the method of storing the oocyte, e.g., by freezing or drying, includes treating the oocyte such that the oocyte enters a dormant state prior to storing the oocyte. This method involves introducing into the oocyte, e.g., microinjecting into the cytoplasm of the oocyte, a protective agent that is substantially non-permeating with respect to mammalian cell membranes and that maintains the viability of the cell such that it can be stored in a temporarily dormant state and substantially restored to an active state. The microinjected oocyte is subjected to conditions that cause it to enter a dormant state and is stored in this dormant state. The stored cell can be subsequently restored to an active state. Low levels, less than or equal to about 6, 5, 4, 3, 2, or 1 M, or even less than about 0.4 M of protective agent can be used in this method. In addition, a sugar alone can be used as the protective agent, or sugar in combination with one or more conventional cryoprotectant, or in combination with other intracellular sugars or extracellular sugars can be used. Preferably, the cytoplasmic concentration of the sugar or sugars is less than 0.3, 0.2, 0.1, 0.05, or 0.01 M after introduction of the protective agent, but before freezing or drying of the cell. In other embodiments, the extracellular concentration of the sugar or sugars is preferably less than 0.3, 0.2, 0.1, 0.05, or 0.01 M after dilution into a liquid medium that includes the oocyte. In other embodiments, the final concentration of sugar (or sugars) in the medium containing the oocyte is at least 2, 3, 4, 5, or 10 fold greater than the cytoplasmic concentration of intracellular sugar (or sugars) after introduction into the oocyte and before freezing or drying of the oocyte. The intracellular and extracellular protective agents can be the same or different molecular species.

Such methods can use one or more of the following sugars as protective agents: monosaccharides, disaccharides, and other oligosaccharides. Preferably, the protective agent is substantially non-permeable such that at least 50, 60, 70, 80, 90, or 95% of the agent does not migrate across the plasma membrane into or out of the cell, by active or passive diffusion. Sugars that can be used include sugars having a glass transition temperature of the maximally freeze-concentrated solution (Tg') that is at least -60°C, -50°C, -40°C, -30°C, -20°C, -10°C, or

0°C. Examples of such sugars are described, e.g., in U.S. Publication Numbers 20020045156 and 20020098470, the contents of which are incorporated herein by reference. The Tg' of other sugars may be routinely determined using standard methods such as those described by Levine and Slade (1998) *J. Chem. Soc., Faraday Trans.* 1, 84:2619-2633. A sugar (or sugars) or conventional cryoprotectant with a Tg' below -50°C can be combined with a sugar (or sugars) with a Tg' above -50°C such that the resulting mixture has a Tg' of at least -60°, -50°, -40°, -30°, -20°, -10°, or 0°C, and this mixture can be used for cryopreservation.

Examples of suitable monosaccarides for use in the methods include those that have an aldehyde group (i.e., aldoses) or a keto group (i.e., ketoses). Monosaccharides can be linear or cyclic, and they can exist in a variety of conformations. Other sugars include those that have been modified (e.g., wherein one or more of the hydroxyl groups are replaced with halogen, alkoxy moieties, aliphatic groups, or are functionalized as ethers, esters, amines, or carboxylic acids). Examples of modified sugars include α- or β-glycosides such as methyl α-D-glucopyranoside or methyl β-D-glucopyranoside; N-glycosylamines; N-glycosides; D-gluconic acid; D-glucosamine; D-galactosamine; and N-acetyl-D-glucosamine. In other embodiments, the protective agent can be an oligosaccharide that includes at least 10, 25, 50, 75, 100, 250, 500, 1000, or more monomers. The oligosaccharide can consist of identical monomers or a combination of different monomers. Other suitable oligosaccharides include: hydroxyl ethyl starch, dextran, cellulose, cellobiose, and glucose. Other suitable protective agents include one or more of: compounds that contain a sugar moiety and that may be hydrolytically cleaved to produce a sugar; glycoproteins and glycolipids, e.g., that preferably have been modified by the addition of 1, 2, 3, 4, 5 or more sugar moieties derived from sugars with a Tg' of at least -60°, -50°, -40°, -30°, -20°, -10°, or 0°C; and glycoproteins and glycolipids with a molecular weight of at least 120 daltons. By "sugar moiety" is meant a protective sugar that includes a group that can be bonded to another compound. For example, a reactive group--such as an alcohol, primary amine, or secondary amine--in a sugar can react with a compound, forming a product that includes the sugar moiety. Another suitable extracellular preservation agent is a lectin or any protein that can non-covalently or covalently bind to a sugar that forms part of a cell-surface glycoprotein or glycolipid. This binding may stabilize the cellular membrane during storage of the cell.

Examples of other cyroprotectants that may be used in the methods include sugars, polyols, glycosides, polymers, and soluble proteins with a molecular weight of at least 120 daltons.

After treatment with the microinjected protective agent and, optionally, the external protective agent, the oocyte is then prepared for storage. In general, the oocyte may be prepared for storage by freezing and/or drying. Plunge freezing, vacuum drying, air drying, as well as freeze drying techniques may be employed. Typically, oocytes are cooled at a rate of 0.1 to 10° C/minute, preferably between 0.3° and 5° C/minute or between 0.5° and 2° C/minute. In some embodiments, the oocytes are cooled at a rate between 0.1° and 200° C/minute, preferably between 0.5° and 100° C/minute, between 1° and 10°C/minute, or 10°C and 5° C/minute, inclusive. The oocytes are cooled to a final temperature of at least -60°, -50°, -40°, -30°, -20°, -10°, 0°, 10°, or 20° C.

Once the oocyte is prepared for storage, it is stored in a manner appropriate to its preparation. Frozen oocytes can be stored at cryogenic temperatures and dried oocytes can be dry stored at ambient or other temperatures as appropriate.

Recovery of stored oocytes is geared to the method of their preparation for storage. Dried oocytes are rehydrated, and frozen oocytes are thawed. Preferably, at least 25, 35, 50, 60, 70, 80, 90, 95, or 100% of the recovered cells are viable. Cell viability may be measured using any standard assay, such as a "live/dead" assay using the green dye calcein-AM to indicate viable cells and the red dye ethidium homodimer to indicate dead cells, according to the manufacturer's protocol (Molecular Probes, Inc.). Thus, in some embodiments, the methods of the invention further include determining the viability of the stored oocyte. In another embodiment, at least 5, 10, 15, 25, 35, 50, 60, 70, 80, 90, or 95% of the recovered oocytes may be fertilized, e.g., using standard *in vitro* fertilization techniques (see, for example, Summers et al., Biol. Reprod. 53:431-437, 1995).

Methods of preparing oocytes for storage and methods of storing oocytes and recovering stored oocytes are described, e.g., in U.S. Publication Numbers 20020045156 and 20020098470, the contents of which are incorporated herein by reference. Additional methods for freezing or cryopreservation of oocytes can be found in Gook, D.A. & Edgar, D.H. (1999) Human Reprod

14:2938-40; Kuleshova, L. et al. (1999) Human Reprod 14:3077-9; Lanzendorf, S.E. et al. (1999) Fertility and Sterility 71:575-7; Polak de Fried, E. et al. (1998) Fertility and Sterility 69:555-7; Porcu, E. et al. (1998) Proceedings XVI World Congress Fertility and Sterility, San Francisco, 4-9 Oct 1998, Eds: Kempers RD, Cohen J, Haney AF, Younger JB, pp599-613; and Fabbri, R. et al. (2001) Human Reprod. 16:411-6.

Harvested oocytes may be stored for relatively short periods of times in a medium. Short period storage of oocytes includes periods ranging from less than an hour to a few days. Preferably, the harvested oocyte is transferred to a medium containing hyaluronidase to remove additional cells, the oocyte is washed, and then stored for three to four hours, at 37° C under 5% Carbon Dioxide. Storage media may include one of the following: BM1 media (NMS Bio-Medical, Praroman, Switzerland), Bicarbonate buffered CZB media or Hepes buffered CZB media (both at pH 7.4) (CZB buffer is described in Chatot, CL et. al. Development of 1-cell embryos from different strains of mice in CZB medium Biol. Reprod. 42:432-440), and finally another medium that maybe used is Human Tubal Fluid (HTF) medium or modified HTF medium.

<u>*In Vitro* Fertilization</u>

As provided herein, the methods are useful in evaluating the quality of an unfertilized oocyte, e.g., to produce an embryo that can develop to term and/or is unliely to be affected by a specific genetic disorder. Such methods allow for the storage of higher quality oocytes and the use of those oocytes in reproductive treatments such as *in vitro* fertilization. Accordingly, the methods of the invention can include fertilizing the stored oocyte.

Methods of providing an *in vitro* fertilized embryo are known in the art. Several of these techniques are described below.

Sperm insemination

Upon reactivation of a stored oocyte, a sperm sample can be obtained and prepared to select out the most motile sperm. The sperm sample can also be obtained in advance and maintained under conditions, e.g., cyropreserved, such that the sperm remain viable.

Motile sperm are placed next to the egg in a culture dish and the culture dish is placed in an incubator. The next day the eggs can be examined to determine whether fertilization has occurred.

Intracytoplasmic sperm injection (ICSI)

This is a technique that involves the injection of a single sperm directly into the oocytes. This procedure has been used in couples who have no fertilization following a previous *in vitro* fertilization cycle or in cases of severe male factor. After the granulosa cells have been stripped away from the oocytes with enzymes, the oocyte is held in place by a holding pipette. The other pipette which is much smaller and sharper is used to pick up a single sperm. The smaller pipette is then brought into proper position and then inserted through the zona pellucida and into the cytoplasm of the oocytes where the sperm is injected.

Implantation

In some aspects, the methods of the invention can further include implanting the embryo, e.g., the embryo produced by the methods described herein, into a female recipient. Various methods of implanting human *in vitro* produced embryos are known. An example of one of these methods is described below.

Human Embryo transfer

If fertilization has resulted, then the embryo transfer is performed usually 72 hours after the egg retrieval. The catheter is passed through the cervix to approximately 1 cm below the uterine fundus. The depth of transfer may be judged from either a previous trial transfer, by measuring its length from external markings or by gently touching the uterine fundus and withdrawing 1 cm. Embryos are loaded in the distal end of the transfer catheter and are gently

injected using a small tuberculin syringe. After transfer the catheter is checked for retained embryos under a dissecting microscope.

Examples

Example 1: Test for aneuploid

Polar body biopsy is performed 1 hour after ovum pick-up and the polar body is fixed immediately. After fixation, the polar bodies are analyzed by fluorescence *in situ* hybridization (FISH) using probes for 13, 16, 18, 21, and 22 labelled respectively in SpectrumRed, SpectrumAqua, SpectrumBlue, SpectrumGreen, and SpectrumGold)Vysis Inc., Downers Grove, IL).

The slide with the fixed polar bodies and the probes are co-denatured on a hotplate at 73°C for 5 minutes and then hybridized at 37°C for 3 hours. The excess probe is washed for 5 minutes at 73C in 0.7X SSC solution, and then the slides are mounted in antifade solution.

The slides are observed with a fluorescence microscope with filters for the respective fluorochromes. After FISH analysis, the eggs are frozen as described previously.

Example II: Screening for genetic disease

The isolated polar body is lysed by incubating it at 65°C for 10 min. After lysis the PCR tubes are immediately put on ice. Two consecutive PCR rounds are performed in all multiplex PCR. The PCR program, concentrations of primers, and the amount of PCR products used in second rounds depend on the genetic disease to be diagnosed and markers used.

The final PCR products (3 μl) are mixed with 3 μl of loading buffer (0.0125g bromophenol blue, 2 ml glycerol, 3 ml water) and loaded onto an ALFExpress Automated DNA Sequencer from Amersham Pharmacia Biotech (Roosendaal, The Netherlands). Results are processed using Allelelinks software provided by the manufacturer.

A number of embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the invention. Accordingly, other embodiments are within the scope of the following claims.